Gut power: Modulation of human amyloid formation by amyloidogenic proteins in the gastrointestinal tract

Citation for the original published paper (version of record):
Wittung Stafshede, P. (2022). Gut power: Modulation of human amyloid formation by amyloidogenic proteins in the
gastrointestinal tract. Current Opinion in Structural Biology, 72: 33-38.
http://dx.doi.org/10.1016/j.sbi.2021.07.009

Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Structural Biology

Gut power: Modulation of human amyloid formation by amyloidogenic proteins in the gastrointestinal tract

Pernilla Wittung-Stafshede

Abstract

Protein assembly into amyloid fibers underlies many neurodegenerative disorders. In Parkinson's disease, amyloid formation of α-synuclein is linked to brain cell death. The gut–brain axis plays a key role in Parkinson's disease, and initial α-synuclein amyloid formation may occur distant from the brain. Because different amyloidogenic proteins can cross-seed, and α-synuclein is expressed outside the brain, amyloids present in the gut (from food products and secreted by microbiota) may modulate α-synuclein amyloid formation via direct interactions. I here describe existing such data that only began to appear in the literature in the last few years. The striking, but limited, data set—spanning from acceleration to inhibition—calls for additional investigations that may unravel disease mechanisms as well as new treatments.

Addresses

Department of Biology and Biological Engineering, Chalmers University of Technology, S-41296, Gothenburg, Sweden

Corresponding author: Wittung-Stafshede P (pernilla.wittung@chalmers.se)

Current Opinion in Structural Biology 2022, **72**:33–38

This review comes from a themed issue on **Folding and Binding**

Edited by **Fabrizio Chiti** and **Anna Sablina**

For a complete overview see the Issue and the Editorial

https://doi.org/10.1016/j.sbi.2021.07.009

Keywords

Parkinson's disease, Alpha-synuclein, Cross-reactivity, Functional amyloids, Microbiome.

Introduction

Today we know of many human diseases, often involving neurodegeneration, that are based on protein aggregation into amyloid fibers [1]. Amyloid fibers all share a common cross-β structure [2], but they can be formed by numerous different proteins with different starting structures. Parkinson's disease (PD) [3,4] is the second most common neurodegenerative disorder, after Alzheimer's disease, among those involving amyloid fibril formation. The number of patients in the world with neurodegenerative disorders, such as PD, is increasing, but we have no cures: there are only symptomatic drugs that provide temporary reliefs. PD is characterized by widespread deterioration of dopaminergic neurons in the substantia nigra. The molecular pathology of PD is directly linked to the assembly of the protein α-synuclein (aS) into amyloid fibrils; such aS amyloids are the major components of pathological inclusions, so-called Lewy bodies, found in brains of patients with PD [5]. The 140-residue aS monomer, intrinsically disordered in solution but helical when bound to lipid vesicles, is believed to function in vesicle fusion and transport in presynaptic nerve termini [6]. Aberrant assembly of aS monomers to amyloid fibers is thought to be a toxic process that is coupled to mitochondrial dysfunction, oxidative stress, protein degradation failure, and eventually cell death [7]. Soluble aS oligomers have been proposed to be most dangerous [8], but studies have also demonstrated that amyloid fibrils are toxic and can be transmitted from cell to cell [9,10]. Recent *in vitro* results showed that oligomers are not only intermediates on the path to amyloid fibers; preformed aS amyloid fibers can also release oligomeric species that are toxic to neurons [11]. An important yet unanswered question is what cellular processes, imbalances, or external species initiate the cascade of deleterious reactions that result in amyloid formation and neurodegeneration. In this respect, it is important to point out that in addition to neuronal cells, aS is expressed in many cell types in the body including enteroendocrine cells (EECs) of the gut epithelium. The latter is perhaps most relevant as the gut–brain axis has been emphasized as a central 'highway' in the spreading of PD.

The role of the gut in PD

It was recently speculated that the gut microbiome can initiate as well as modulate PD [12,13], and studies have indicated roles for the gastrointestinal tract and the enteric nervous system (ENS) [14] (Figure 1). ENS neurons are found in the walls of the gastrointestinal tract and connect directly with EECs which, in turn, communicate with the gut content. Recent studies have shown that Lewy bodies are often observed in ENS neurons in early stages of PD, introduction of preformed aS amyloid fibers in the gastrointestinal tract of mice induces Lewy body formation in the brain [15], and the

Figure 1

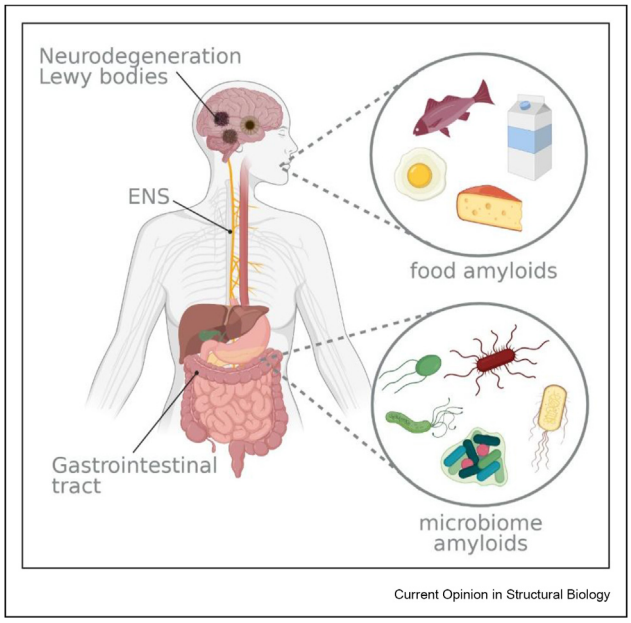


Illustration of the gut–brain axis. The enteric nervous system (ENS) connects the brain, where amyloid deposits are found in, for example, Parkinson's disease, with the gastrointestinal tract. Note that the ENS extends from the esophagus all the way to the anus (here hidden in view by the gastrointestinal tract). Both nondegraded food amyloids and amyloids secreted by the microbiome may interact with human amyloidogenic proteins in neuronal cells connected to the gut. The picture was made with BioRender.com.

risk of PD was drastically reduced for patients with truncal vagotomy [16].

Although the molecular determinants are unknown [17], studies have shown that amyloids of one protein can cross-seed other amyloidogenic proteins, both among different human proteins [18] and between human and nonhuman amyloidogenic proteins [19**]. The latter is interesting with respect to the gut, as the microbiome contains several bacteria that secrete amyloids as part of their biofilms and food products sometimes contain amyloids (Figure 1). Several food proteins that act as allergens (such as whey and casein proteins in milk, ovalbumin and lysozyme in egg, and β-parvalbumin in fish) adopt amyloid states that confer protection against gastrointestinal digestion and allow uptake in the body [20]*. One may imagine that nonhuman amyloids in the gut may cross-react and trigger amyloid formation of aS in nearby cells that then transfer to the brain or that bacterial/food amyloids themselves travel via blood or the ENS to the brain and interact with aS polypeptides there. After a first section on the link to the innate immune system, I will summarize the current knowledge of direct interactions between aS and amyloids from bacteria and diet that can be found in the gut, as well as how such cross-reactivity affects aS amyloid formation kinetics *in vitro* (Table 1). Although there are only a few such studies reported to date, key discoveries have been made.

Modulations via the immune system

In addition to activities in brain function, aS is thought to be a player in the innate immune system. In response to viral and bacterial infections, ENS cells trigger increased expression of aS [21]. Microbes and viruses reaching the gut via the diet, or other insults to the gut, may thus promote aS overexpression in the ENS as a protective immune response. However, this may result in aS amyloid formation because of high local protein concentration, perhaps coupling with defective protein aggregate clearance. aS amyloids formed in the ENS may then travel to the brain and trigger PD. Most nonhuman amyloid structures can trigger an immune response that leads to weakening of the gut barrier [22]. In addition, aging, a major risk factor for PD, increases the permeability of the intestine [23]. Such changes will allow for increased uptake of nonhuman amyloids (and other bacterial and viral diet components) from the gut that can then promote PD (and amyloid formation) in at least two ways: via an immune response that upregulates aS expression and by direct interactions (in EECs or the ENS) between the nonhuman proteins and aS [24].

Cross-reactivity with microbiome amyloidogenic proteins

Many microorganisms produce extracellular biofilms that help the organism to adhere to surfaces, for example, in the gastrointestinal tract [25,26]. Such biofilms contain amyloid fibers, and in *Escherichia coli* and other bacteria, CsgA is the protein that assembles to amyloids (often called curli) [27]. The bacterial genome codes for several accessory proteins that control the production of curli fibers and assure they only form extracellularly [28]. CsgA contains five peptide repeat units, each predicted to form a β-loop-β motif, that are proposed to constitute the core of the amyloid fibers [29]. An initial study showed that when rats were fed curli-producing *E. coli*, there was an increase in aS amyloid formation as compared with animals that had been fed curli-deficient *E. coli* [13]. In subsequent work, it was observed that the bacterial composition of the microbiome, and/or products produced by the bacteria, in aS-expressing mice could increase aS brain deposits, neuroinflammation, and motor deficits [12]. Thus, alterations in the microbiome appear as a risk factor for PD. To search for a possible molecular mechanism that involved direct cross-reactivity, *in vitro* experiments with purified CsgA and aS were executed [30**]. Not only did CsgA preformed amyloids accelerate aS amyloid formation but also the monomeric form of CsgA at substoichiometric amounts (at low enough concentration such that CsgA alone did not aggregate) accelerated aS

Table 1

Consequences for *in vitro* aS amyloid formation on interaction with nonhuman amyloidogenic proteins that may be found as amyloids in the gut.

Protein	Source	Structure	Effect	Year	Reference
CsgA	*E. coli*		Acceleration	2020	[30]
FapC	*Pseudomonas*		No effect	2019	[32]
FapC truncation			Inhibition	2019	[32]
β-parvalbumin	Fish		Inhibition	2018	[36]
Lysozyme	Egg		Acceleration	2021	[38]
β-lactoglobulin	Milk		No effect	2021	[38]

While CsgA and FapC are unstructured polypeptides that readily assemble to amyloids, β-parvalbumin, lysozyme, and β-lactoglobulin have globular structures when functional.

amyloid formation *in vitro*. Because the amount of added CsgA was sub-stoichiometric, a catalytic mechanism involving transient interactions was proposed, and this conclusion agreed with lack of stable interactions found in surface plasmon resonance binding experiments [30**]. The cross-reactivity between CsgA and aS depended on CsgA's amyloid-forming ability as aS-expressing mice mono-colonized with *E. coli* bacteria which produced an amyloid-deficient CsgA protein (or mice injected with nonamyloidogenic CsgA) did not show much aS aggregation and had less motor impairment as compared with mice with wild-type CsgA [30**]. Interestingly, two curli operon proteins acting as chaperones for CsgA inside cells (CsgC and CsgE) also affected aS amyloid formation: CsgC accelerated, whereas CsgE inhibited, aS amyloid formation via differential transient interactions with the C-terminal of aS [31].

Like CsgA, *Pseudomonas aeruginosa* FapC assembles into amyloids that provide structural stability to the biofilm, but this protein has only three amyloid-promoting peptide repeat units. To elucidate the role of these repeat units in FapC amyloid formation, various variants of FapC were characterized *in vitro*. Surprisingly, it was found that despite removal of all repeats, the protein still formed amyloids, albeit slower. The delay in amyloid formation kinetics allowed for disulfide-bond formation between cysteine residues in FapC monomers, which in turn delayed the aggregation process further [32**]. Like *E. coli*, *Pseudomonas* is abundant in the gut [33], and cross-reactivity between FapC and aS was tested *in vitro*. In contrast to CsgA, preformed amyloids of neither wild-type nor mutant FapC affected aS amyloid formation [32**]. However, the slower-aggregating variant of FapC was found to interact with aS (at monomeric or oligomeric states) in a process that retarded aS amyloid formation *in vitro*; instead, dead-end oligomers containing both proteins were detected [32**].

Cross-reactivity with food protein amyloids

The most common allergen in fish, the highly abundant protein β-parvalbumin (PV), forms amyloids that escape gastrointestinal degradation and transit to the blood [34,35]. PV is a small, calcium-binding protein with a helical structure that transforms to amyloids on calcium removal. Because fish is generally considered beneficial toward age-related diseases such as dementia and Alzheimer's disease, we speculated that an explanation

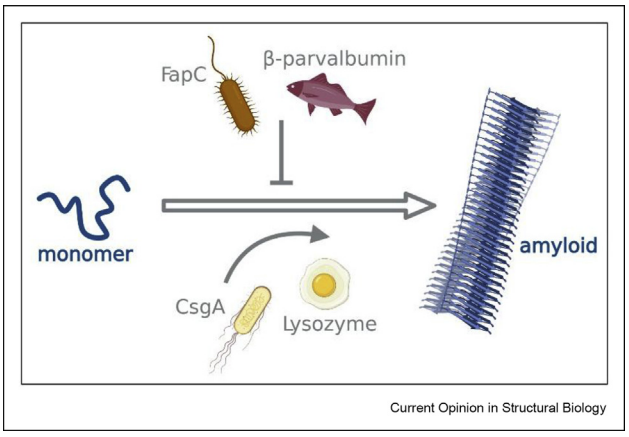
would be cross-reactivity of PV amyloids with human amyloidogenic proteins [36**]. Indeed, *in vitro* experiments showed that PV amyloids readily blocked aS amyloid formation. It was revealed that PV amyloids, perhaps via their protruding negatively charged calcium-binding loops, scavenged aS monomers to the PV amyloid surface. Immunogold staining experiments showed PV amyloids to be covered with aS [36**]. However, on calcium addition to such systems, with the calcium likely binding to the protruding loops on the PV amyloids, aS was released from the PV amyloids and started to aggregate. In addition to cross-reactivity, the experiments demonstrated that only primary (i.e. not secondary nucleation or fragmentation) processes are involved in PV amyloid formation, and on further investigation, the amyloid formation mechanism was shown to involve on-path disulfide bridged dimers [37]*. Notably, inhibition of amyloid formation via scavenging of monomers to a preformed amyloid of a different protein is a new inhibition mechanism not reported before.

In addition to fish, other food products contain protein amyloids that are not fully digested in the gut: for example, whey and casein proteins (milk), ovalbumin, lysozyme and ovotransferrin (egg), bovine serum albumin and hemoglobin (blood), and glutelin (wheat) [20]. However, there are few cross-reactivity studies between such proteins and human amyloidogenic proteins. In a recent study, it was shown that amyloids of egg lysozyme, but not amyloids of bovine β-lactoglobulin, accelerated aS amyloid formation *in vitro*. For lysozyme, the authors demonstrated a surface-mediated seeding mechanism that depended on favorable electrostatic interactions between aS and lysozyme [38**].

Conclusions and outlook

Microbial and food amyloids can modulate aS amyloid formation via direct interactions, and this type of cross-reactivity, in addition to immune system effects, may contribute to the gut–brain 'highway' in PD (Figure 2). In addition to *Escherichia* and *Pseudomonas* already mentioned when discussing CsgA and FapC, *Streptococcus*, *Staphylococcus*, *Salmonella*, *Mycobacteria*, *Klebsiella*, *Citrobacter*, and *Bacillus* bacterial species (and many others) are found in the gut, and these also make extracellular amyloids. Although little is known about the abundance of different bacterial amyloids because of extensive variability of composition from person to person, it is important to note that for cross-seeding, only small amounts of seeds are needed. Food amyloids will likely become more common in the future as amyloid structures have favorable foaming, emulsifying, and gel properties that improve food texture [39]. Proteins forced into amyloid structures can be used, for example, as thickening ingredients, surface active media, carriers for nutrients and drugs, packaging materials, and

Figure 2



Scheme showing reported consequences (inhibition or acceleration) on α-synuclein amyloid formation *in vitro* upon direct interactions with food (fish β-parvalbumin and egg lysozyme) and bacterial (*E. coli* CsgA and *Pseudomonas* FapC variant) amyloidogenic proteins. (Milk β-lactoglobulin amyloids, also tested, had no effect.) The picture was made with BioRender.com.

antimicrobial substances. Most proteins in our diet are digested to amino acids in the gut, but studies show that amyloids can be resistant to gastric conditions [39]. Before using amyloids in food production, one should assess possible toxicity case by case. Amyloidogenic proteins in food that escape degradation may both accelerate and inhibit human amyloidogenic proteins. Moreover, food amyloids may cross-react with biofilm amyloids and thereby affect the microbiota composition.

Many more *in vitro* experiments with purified proteins that probe direct interactions between human and gut amyloidogenic proteins are desired. Knowledge of such interactions and consequences *in vitro* will complement *in vivo* studies that, when taken together, may reveal underlying (causative) reasons for the onset of human amyloid diseases such as PD, as well as unravel novel avenues toward treatments supplied via the gut. Notably, there are already clinical trials showing promising results for orally administered drugs (e.g. squalamine and levodopa) that appear to modulate aS aggregation and propagation in the ENS [21,40]. In fact, several aminosterol derivatives, such as squalamines and trodusquemine, are currently explored as modulators of cell membrane physicochemical properties [41,42]. The therapeutic strategy here is to stabilize cell membranes (possibly in the ENS) so they become resistant to perturbations by protein aggregates (e.g. aS oligomers and amyloids) associated with neurodegeneration.

I want to end with a timely connection to the COVID-19 pandemic. It has been proposed that a peptide from the COVID-19 spike protein, created during virus

processing, can assemble into amyloids. Because COVID-19 may infect the gut [43] in addition to the lungs and other organs, cross-reactivity between this virus-derived amyloid and human amyloidogenic proteins in the gut may underlie observed post-infection neurodegenerative symptoms [44]*.

Conflict of interest statement

Nothing declared.

Acknowledgments

The author thanks the Knut and Alice Wallenberg Foundation and the Swedish Research Council for funding.

References

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

1. Chiti F, Dobson CM: **Protein misfolding, amyloid formation, and human disease: a summary of progress over the last decade**. *Annu Rev Biochem* 2017, **86**:27–68.
2. Li B, Ge P, Murray KA, Sheth P, Zhang M, Nair G, Sawaya MR, Shin WS, Boyer DR, Ye S, Eisenberg DS, Zhou ZH, Jiang L: **Cryo-EM of full-length α-synuclein reveals fibril polymorphs with a common structural kernel**. *Nat Commun* 2018, **9**:3609.
3. John A, van der Pluijm W: **The global prevalence of Parkinson's disease over the next ten years**. *Ann Neurol* 2018, **84**. S219-S219.
4. Elkouzi A, Vedam-Mai V, Eisinger RS, Okun MS: **Emerging therapies in Parkinson disease - repurposed drugs and new approaches**. *Nat Rev Neurol* 2019, **15**:204–223.
5. Stefanis L: **α-Synuclein in Parkinson's disease**. *Cold Spring Harbor Perspect Med* 2012, **2**.
6. Burré J, Sharma M, Südhof TC: **Cell biology and pathophysiology of α-synuclein**. *Cold Spring Harbor Perspect Med* 2018, **8**.
7. De Mattos EP, Wentink A, Nussbaum-Krammer C, Hansen C, Bergink S, Melki R, Kampinga HH: **Protein quality control pathways at the crossroad of synucleinopathies**. *J Parkinsons Dis* 2020, **10**:369–382.
8. Gosavi N, Lee HJ, Lee JS, Patel S, Lee SJ: **Golgi fragmentation occurs in the cells with prefibrillar alpha-synuclein aggregates and precedes the formation of fibrillar inclusion**. *J Biol Chem* 2002, **277**:48984–48992.
9. Peelaerts W, Bousset L, Van der Perren A, Moskalyuk A, Pulizzi R, Giugliano M, Van den Haute C, Melki R, Baekelandt V: **alpha-Synuclein strains cause distinct synucleinopathies after local and systemic administration**. *Nature* 2015, **522**: 340–344.
10. Pieri L, Madiona K, Bousset L, Melki R: **Fibrillar alpha-synuclein and huntingtin exon 1 assemblies are toxic to the cells**. *Biophys J* 2012, **102**:2894–2905.
11. Cascella R, Chen SW, Bigi A, Camino JD, Xu CK, Dobson CM, Chiti F, Cremades N, Cecchi C: **The release of toxic oligomers from α-synuclein fibrils induces dysfunction in neuronal cells**. *Nat Commun* 2021, **12**:1814.
12. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, Ilhan ZE, Challis C, Schretter CE, Rocha S, Gradinaru V, Chesselet MF, Keshavarzian A, Shannon KM, Krajmalnik-Brown R, Wittung-Stafshede P, Knight R, Mazmanian SK: **Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease**. *Cell* 2016, **167**. 1469-1480 e12.
13. Chen SG, Stribinskis V, Rane MJ, Demuth DR, Gozal E, Roberts AM, Jagadapillai R, Liu R, Choe K, Shivakumar B, Son F, Jin S, Kerber R, Adame A, Masliah E, Friedland RP: **Exposure to the functional bacterial amyloid protein curli enhances alpha-synuclein aggregation in aged fischer 344 rats and Caenorhabditis elegans**. *Sci Rep* 2016, **6**:34477.
14. Chandra R, Hiniker A, Kuo Y-M, Nussbaum RL, Liddle RA: **α-Synuclein in gut endocrine cells and its implications for Parkinson's disease**. *JCI Insight* 2017, **2**.
15. Uemura N, Yagi H, Uemura MT, Hatanaka Y, Yamakado H, Takahashi R: **Inoculation of alpha-synuclein preformed fibrils into the mouse gastrointestinal tract induces Lewy body-like aggregates in the brainstem via the vagus nerve**. *Mol Neurodegener* 2018, **13**:21.
16. Liu B, Fang F, Pedersen NL, Tillander A, Ludvigsson JF, Ekbom A, Svenningsson P, Chen H, Wirdefeldt K: **Vagotomy and Parkinson disease: a Swedish register-based matched-cohort study**. *Neurology* 2017, **88**:1996–2002.
17. Daskalov A, Martinez D, Coustou V, El Mammeri N, Berbon M, Andreas LB, Bardiaux B, Stanek J, Noubhani A, Kauffmann B, Wall JS, Pintacuda G, Saupe SJ, Habenstein B, Loquet A: **Structural and molecular basis of cross-seeding barriers in amyloids**. *Proc Natl Acad Sci U S A* 2021, **118**.
18. Oskarsson ME, Paulsson JF, Schultz SW, Ingelsson M, Westermark P, Westermark GT: **In vivo seeding and cross-seeding of localized amyloidosis: a molecular link between type 2 diabetes and Alzheimer disease**. *Am J Pathol* 2015, **185**: 834–846.
19. ** Werner T, Horvath I, Wittung-Stafshede P: **Crosstalk between alpha-synuclein and other human and non-human amyloidogenic proteins: consequences for amyloid formation in Parkinson's disease**. *J Parkinsons Dis* 2020, **10**:819–830.
Recent review summarizing avaliable data for protein–protein cross-reactivity, with consequences on aS amyloid formation, for human and non-human amyloidogenic proteins.
20. * Cao Y, Mezzenga R: **Food protein amyloid fibrils: origin, structure, formation, characterization, applications and health implications**. *Adv Colloid Interface Sci* 2019, **269**: 334–356.
A comprehensive review about the presence of amyloid fibers in food with focus on origin, transit in the gastrointestinal tract, biotechnological applications, as well as possible health concerns.
21. Barbut D, Stolzenberg E, Zasloff M: **Gastrointestinal immunity and alpha-synuclein**. *J Parkinsons Dis* 2019, **9**:S313–S322.
22. Miller AL, Bessho S, Grando K, Tukel C: **Microbiome or infections: amyloid-containing biofilms as a trigger for complex human diseases**. *Front Immunol* 2021, **12**:638867.
23. Friedland RP, Chapman MR: **The role of microbial amyloid in neurodegeneration**. *PLoS Pathog* 2017, **13**, e1006654.
24. Killinger BA, Labrie V: **Vertebrate food products as a potential source of prion-like alpha-synuclein**. *NPJ Parkinsons Dis* 2017, **3**:33.
25. Macfarlane S, Dillon JF: **Microbial biofilms in the human gastrointestinal tract**. *J Appl Microbiol* 2007, **102**:1187–1196.
26. Hori K, Matsumoto S: **Bacterial adhesion: from mechanism to control**. *Biochem Eng J* 2010, **48**:424–434.
27. Chapman MR, Robinson LS, Pinkner JS, Roth R, Heuser J, Hammar M, Normark S, Hultgren SJ: **Role of Escherichia coli curli operons in directing amyloid fiber formation**. *Science* 2002, **295**:851–855.
28. Evans ML, Chorell E, Taylor JD, Aden J, Gotheson A, Li F, Koch M, Sefer L, Matthews SJ, Wittung-Stafshede P, Almqvist F, Chapman MR: **The bacterial curli system possesses a potent and selective inhibitor of amyloid formation**. *Mol Cell* 2015, **57**: 445–455.
29. Wang X, Hammer ND, Chapman MR: **The molecular basis of functional bacterial amyloid polymerization and nucleation**. *J Biol Chem* 2008, **283**:21530–21539.
30. ** Sampson TR, Challis C, Jain N, Moiseyenko A, Ladinsky MS, Shastri GG, Thron T, Needham BD, Horvath I, Debelius JW, Janssen S, Knight R, Wittung-Stafshede P, Gradinaru V, Chapman M, Mazmanian SK: **A gut bacterial amyloid promotes**

alpha-synuclein aggregation and motor impairment in mice. *Elife* 2020, **9**.
Using a mice model that expressed human aS, in combination with *in vitro* experiments with purified proteins, it was shown that amyloids from *E. coli* biofilms promoted aS aggregation and caused motor impairment in these mice.

31. Chorell E, Andersson E, Evans ML, Jain N, Gotheson A, Aden J, Chapman MR, Almqvist F, Wittung-Stafshede P: **Bacterial chaperones CsgE and CsgC differentially modulate human alpha-synuclein amyloid formation via transient contacts**. *PloS One* 2015, **10**, e0140194.

32. ** Christensen LFB, Jensen KF, Nielsen J, Vad BS, Christiansen G, Otzen DE: **Reducing the amyloidogenicity of functional amyloid protein FapC increases its ability to inhibit alpha-synuclein fibrillation**. *ACS Omega* 2019, **4**:4029–4039.
The mechanism of FapC amyloid formation was investigated and it was found that when the reaction was retarded (by deletion of peptide repeat regions) cysteine residues in the protein formed inter-protein disulfides and such species were able to block aS amyloid formation via direct interactions.

33. Monstein H-J, Tiveljung A, Kraft CH, Borch K, Jonasson J: **Profiling of bacterial flora in gastric biopsies from patients with Helicobacter pylori-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis**. *J Med Microbiol* 2000, **49**:817–822.

34. Sanchez R, Martinez J, Castro A, Pedrosa M, Quirce S, Rodriguez-Perez R, Gasset M: **The amyloid fold of Gad m 1 epitopes governs IgE binding**. *Sci Rep* 2016, **6**:32801.

35. Scheers N, Lindqvist H, Langkilde AM, Undeland I, Sandberg AS: **Vitamin B12 as a potential compliance marker for fish intake**. *Eur J Nutr* 2014, **53**:1327–1333.

36. ** Werner T, Kumar R, Horvath I, Scheers N, Wittung-Stafshede P: **Abundant fish protein inhibits alpha-synuclein amyloid formation**. *Sci Rep* 2018, **8**:5465.
Amyloids of PV was found to scavange aS monomers to the amyloid surface, thereby blocking aS amyloid formation. This reaction observed *in vitro* was speculated to contribute to beneficial health properties of fish diets. However, further studies are needed.

37. * Werner TER, Bernson D, Esbjorner EK, Rocha S, Wittung-Stafshede P: **Amyloid formation of fish beta-parvalbumin involves primary nucleation triggered by disulfide-bridged protein dimers**. *Proc Natl Acad Sci U S A* 2020, **117**:27997–28004.
The amyloid formation mechanism of PV was elucidated using biophysical methods, revealing a disulfide-bridged folded dimer as an on-path species.

38. ** Vaneyck J, Segers-Nolten I, Broersen K, Claessens M: **Cross-seeding of alpha-synuclein aggregation by amyloid fibrils of food proteins**. *J Biol Chem* 2021:100358.
Cross-seeding between aS and hen egg white lysozyme and bovine milk β-lactoglobulin was tested *in vitro* resulting in differential effects that were explained in terms of binding affinity and electrostatics.

39. Jansens KJA, Lambrecht MA, Rombouts I, Monge Morera M, Brijs K, Rousseau F, Schymkowitz J, Delcour JA: **Conditions governing food protein amyloid fibril formation—Part I: egg and cereal proteins**. *Compr Rev Food Sci Food Saf* 2019, **18**:1256–1276.

40. Hauser RA, Isaacson SH, Ellenbogen A, Safirstein BE, Truong DD, Komjathy SF, Kegler-Ebo DM, Zhao P, Oh C: **Orally inhaled levodopa (CVT-301) for early morning OFF periods in Parkinson's disease**. *Park Relat Disord* 2019, **64**:175–180.

41. Perni M, Galvagnion C, Maltsev A, Meisl G, Müller MBD, Challa PK, Kirkegaard JB, Flagmeier P, Cohen SIA, Cascella R, Chen SW, Limbocker R, Sormanni P, Heller GT, Aprile FA, Cremades N, Cecchi C, Chiti F, Nollen EAA, Knowles TPJ, Vendruscolo M, Bax A, Zasloff M, Dobson CM: **A natural product inhibits the initiation of α-synuclein aggregation and suppresses its toxicity**. *Proc Natl Acad Sci Unit States Am* 2017, **114**:E1009–E1017.

42. Errico S, Lucchesi G, Odino D, Muscat S, Capitini C, Bugelli C, Canale C, Ferrando R, Grasso G, Barbut D, Calamai M, Danani A, Zasloff M, Relini A, Caminati G, Vendruscolo M, Chiti F: **Making biological membrane resistant to the toxicity of misfolded protein oligomers: a lesson from trodusquemine**. *Nanoscale* 2020, **12**:22596–22614.

43. Brundin P, Nath A, Beckham JD: **Is COVID-19 a perfect storm for Parkinson's disease?** *Trends Neurosci* 2020, **43**:931–933.

44. * Tavassoly O, Safavi F, Tavassoly I: **Seeding brain protein aggregation by SARS-CoV-2 as a possible long-term complication of COVID-19 infection**. *ACS Chem Neurosci* 2020, **11**:3704–3706.
Protein aggregation may be induced by the intact structure of the Covid-19 virus or a peptide derived from the Spike protein predicted to be amyloidogenic. Such interactions may accelerate sporadic neurodegeneration in the infected population.